

Cell Patterning Technology on Polymethyl Methacrylate through Controlled Physicochemical and Biochemical Functionalization

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Supporting Information

S1. PDMS channel specifications

A PDMS channel was fabricated and adhered directly on top to the cell patterns through pressure sensitive adhesive, **Figure S1**.

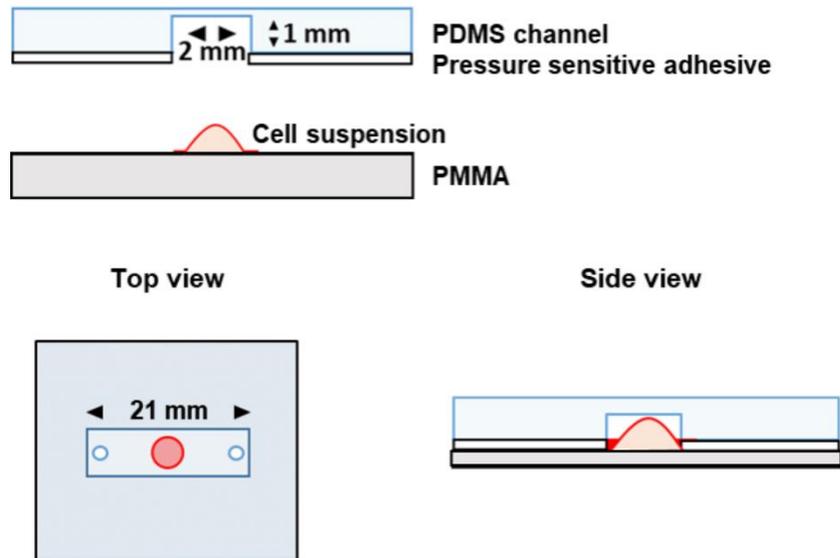


Figure S1. PDMS channel specifications.

S2. PMMA wells specifications

In order to generate wells (2 cm) made of PMMA, three PMMA layers (4 mm) were cut with a laser and pasted both between them and with the glass cover with pressure sensitive adhesive (PSA). **Figure S2**.

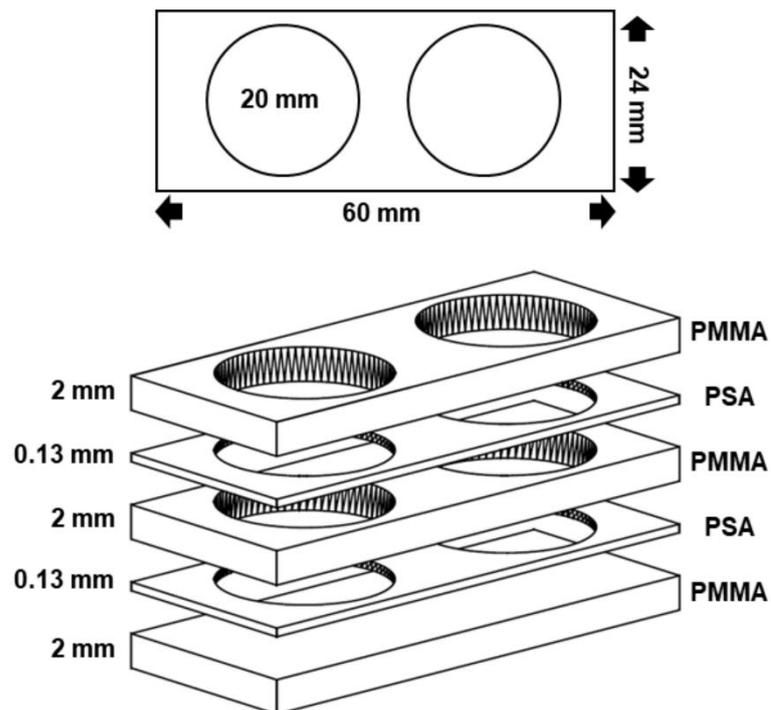


Figure S2. PMMA wells specifications.

S3. Viability assay of cell patterns on PMMA.

Viability of patterned cells was studied directly after patterning (t_0) and after an incubation of 24 h (t_{24}). For this assay, HeLa cells were patterned in arrays of small cell-isles by printing 100 μm fibronectin dots. Patterning protocol and cell adhesion protocol were followed as described in Section 2.4. in the manuscript.

To assess cell viability, a trypan blue assay for patterned cells was followed as previously described [1]. In short, cell culture medium was retrieved from each well either directly after patterning, or after a 24 h incubation at 37 $^{\circ}\text{C}$ and 5 % CO_2 inside a cell culture incubator with serum-free DMEM medium. 4 samples were used for each condition. Afterwards, the samples were washed twice with PBS, and 50% (v/v) of trypan blue in DMEM was added to immediately track their viability. Brightfield images and viability assay were performed with a Corning Cell Counter, Fisher Scientific, Spain. Viability was analyzed using Axion Biosystems Portal.

Viability of patterned cells directly after the patterning protocol (t_0) was 99 %, indicating that the vast majority of the patterned cells were alive within the patterns, which in turn confirms that the patterning protocol does not affect negatively the cells' viability. In the patterns that were incubated for 24 h, a loss in the number of cells could be observed (10 ± 2 and 8 ± 3 cells for the patterns at t_0 and t_{24} , respectively) indicating that between 10 % and 20 % of the patterned cells detach over the course of 24 h. Nevertheless, viability of the patterns at t_{24} was still 98.5 %, indicating that the vast majority of cells that remain in the patterns are alive. Furthermore, cells elongated over the course of the 24 h, which indicate proper adhesion of the cells with the substrate, **Figure S3**.

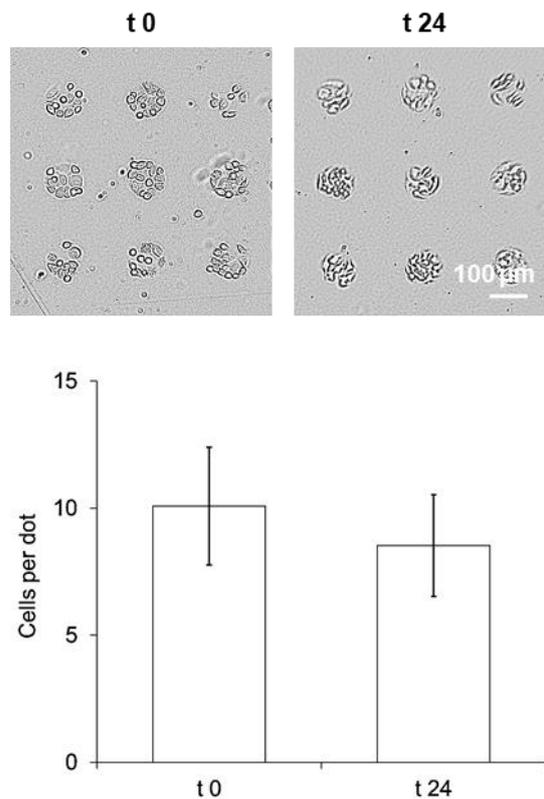


Figure S3. Viability assay of patterned cells. Top; Brightfield images of patterned HeLa cells in 100 μm fibronectin dots after patterning (t_0) and after 24 h incubation (t_{24}). Bottom; Graphic of the number of cells per dot at t_0 and t_{24} . Error bars means SD (180 dots measured from 4 samples).

S4. Printed fibronectin features

For the patterning of cells with different cell-cell contact and cell confluences, different arrays of fibronectin features were printed, **Figure S4**.

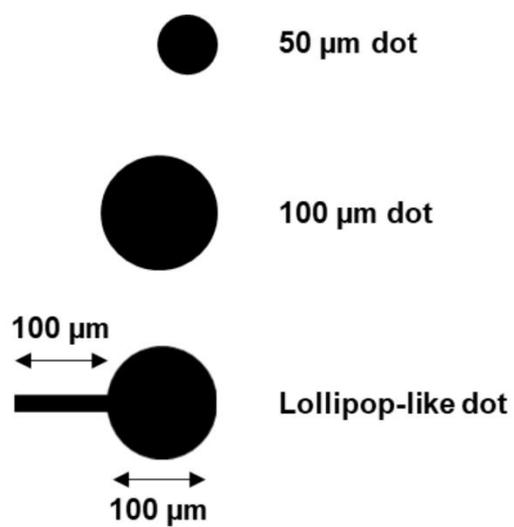


Figure S4. Fibronectin features printed for cell adhesion.

References

- [1] Garcia-Hernando, M.; Calatayud-Sanchez, A.; Etxebarria-Elezgarai, J.; de Pancorbo, M.M.; Benito-Lopez, F.; Basabe-Desmonts, L. Optical Single Cell Resolution Cytotoxicity Biosensor Based on Single Cell Adhesion Dot Arrays. *Anal. Chem.* **2020**, *92*, 9658–9665. <https://doi.org/10.1021/acs.analchem.0c00940>